EXPRESS MAIL NO. EL 755725065US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Christen M. Anderson et al.
Application No.	:	09/809,827
Filed	:	March 16, 2001
For	:	PRODUCTION OF ADENINE NUCLEOTIDE TRANSLOCATOR (ANT), NOVEL ANT LIGANDS AND SCREENING ASSAYS THEREFOR
Art Unit	:	1653
Docket No.	:	660088.420D6
Date	:	May 30, 2001

Commissioner for Patents
Washington, DC 20231

FILING FORMAL DRAWINGS

Commissioner for Patents:

In response to the Notice to File Corrected Application Papers dated May 15, 2001, enclosed are 14 sheets of formal drawings, Figures 1A-19, for filing in the above-identified application.

Respectfully submitted,
Christen M. Anderson et al.
Seed Intellectual Property Law Group PLLC

Stephen J. Rosenman, Ph.D.
Registration No. 43,058

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Enclosures:
Formal Drawings (14 sheets, Figs. 1A-19)

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Fig. 1A

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ANT1m ATCATCAAGATCTTCAAGTCTGATGGCCTGAGGGGGGCTCTACCAGGGTTTCAACGTCTCTGTCCAAGGCATCATTATCTA 557
ANT2m CTGGTTAAGATCTACAAATCTGATGGGATTAAGGGCCTGTACCAGGCTTTAACGTGTCTGTGCAGGGTATTATCATCTA 560
ANT3m CTGGTGAAGATCACCAAGTCCGACGGCATCCGGGGCCTGTACCAGGGCTTCAGTGTCTCCGTGCAGGGCATCATCATCTA 560

ANT1m TAGAGCTGCCTACTTCGGAGTCTATGATACTGCCAAGGGGATGCTGCCTGACCCCAAGAACGTGCACATTTTTGTGAGCT 637
ANT2m CCGAGCCGCCTACTTCGGTATCTATGACACTGCAAAGGGAATGCTTCCGGATCCCAAGAACACTCACATCGTCATCAGCT 640
ANT3m CCGGGCGGCCTACTTCGGCGTGTACGATACGGCCAAGGGCATGCTCCCGGACCCCAAGAACACGCACATCGTGGTGAGCT 640

ANT1m GGATGATTTGCCCAGAGTGTGACGGCAGTCGCAGGGCTGCTGTCCTACCCCTTTGACACTGTTCGTCGTAGAATGATGATG 717
ANT2m GGATGATCGCACAGACTGTCACTGCTGTTGCCGGGTTTGACTTCCTATCCATTTGACACTGTTCGCCGCCGCATGATGATG 720
ANT3m GGATGATCGCGCAGACCGTGACGGCCGTGGCCGGCGTGGTGTCCTACCCCTTCGACACGGTGCGGCGGCGCATGATGATG 720

ANT1m CAGTCCGGCCGGAAAGGGGCCGATTATTATGTACACGGGGACAGTTGACTGCTGGAGGAAGATTGCAAAAGACGAAGGAGC 797
ANT2m CAGTCAGGGCGCAAAGGAACTGACATCATGTACACAGGCACGCTTGACTGCTGGCGGAAGATTGCTCGTGATGAAGGAGG 800
ANT3m CAGTCCGGGCGCAAAGGAGCTGACATCATGTACACGGGCACCGTCGACTGTTGGAGGAAGATCTTCAGAGATGAGGGGGG 800

ANT1m CAAGGCCTTCTTCAAAGGTGCCTGGTCCAATGTGCTGAGAGGCATGGGCGGTGCTTTTGTATTGGTGTTGTATGATGAGA 877
ANT2m CAAAGCTTTTTTCAAGGGTGCATGGTCCAATGTTCTCAGAGGCATGGGTGGTGCTTTTGTGCTTGTCTTGTATGATGAAA 880
ANT3m CAAGGCCTTCTTCAAGGGTGCGTGGTCCAAGGTCCTGCGGGGCATGGGGGGCGCCTTCGTGCTGGTCCTGTACGACGAGC 880

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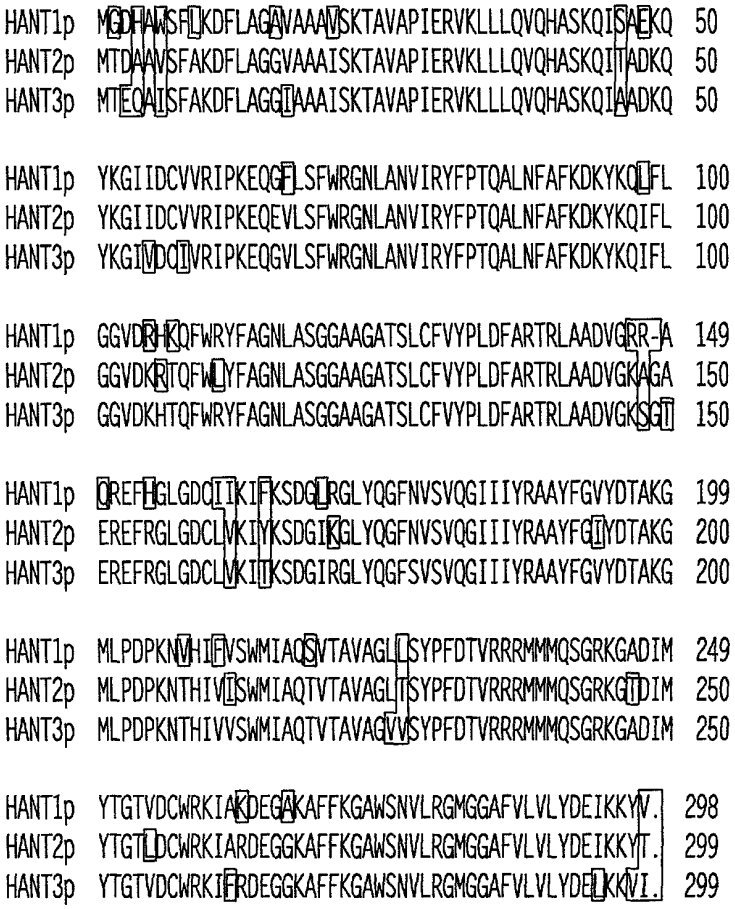
Fig. 1B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HANT2p YKGIIDCVVRIPKEQEVLSFWRGNLANVIRYFPTQALNFAFKDKYKQIFL 100
HANT3p YKGIVDCIVRIPKEQGVLSFWRGNLANVIRYFPTQALNFAFKDKYKQIFL 100
HANT1p GGVDRHKQFWRYFAGNLASGGAAGATSLCFVYPLDFARTRLAADVGRR-A 149
HANT2p GGVDKRTQFWLYFAGNLASGGAAGATSLCFVYPLDFARTRLAADVGKAGA 150
HANT3p GGVDKHTQFWRYFAGNLASGGAAGATSLCFVYPLDFARTRLAADVGKSGT 150
HANT1p QREFHGLGDQIIKIFKSDGLRGLYQGFNVSVQGIIIYRAAYFGVYDTAKG 199
HANT2p EREFRGLGDCLVKIYKSDGIKGLYQGFNVSVQGIIIYRAAYFGIYDTAKG 200
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HANT2p MLPDPKNTHIVISWMIAQTVTAVAGLTSYPFDTVRRRMMMQSGRKGTDIM 250
HANT3p MLPDPKNTHIVVSWMIAQTVTAVAGVVSYPFDTVRRRMMMQSGRKGADIM 250
HANT1p YTGTVDCWRKIAKDEGAKAFFKGAWSNVLRGMGGAFVLVLYDEIKKYV. 298
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Fig. 2

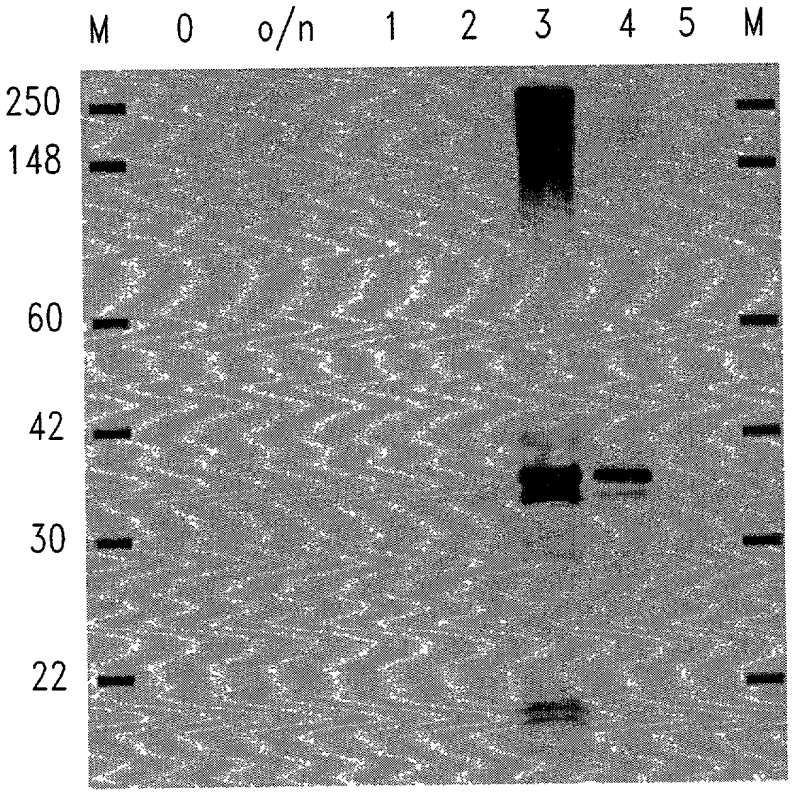

Fig. 3

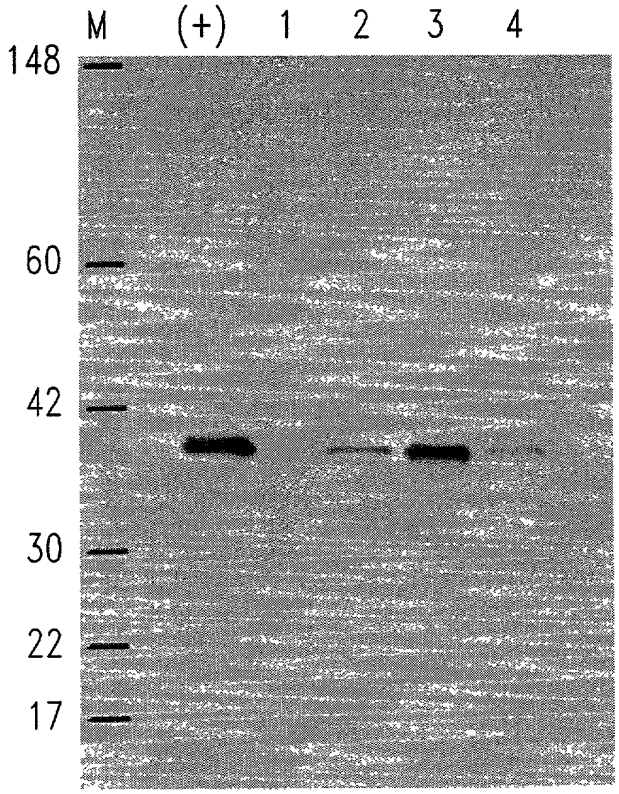

Fig. 4

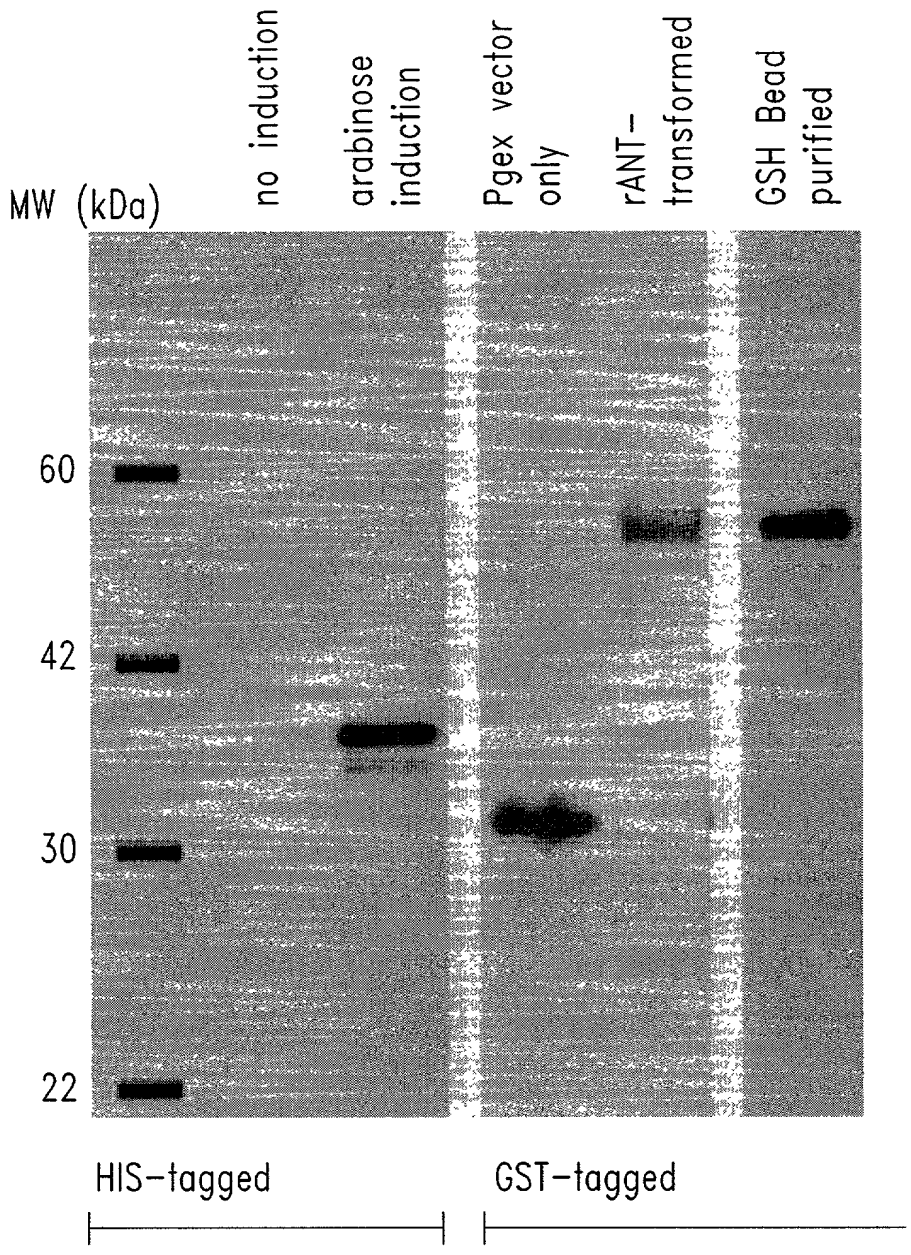
MW (kDa)
no induction
arabinose induction
Pgex vector only
rANT-transformed
GSH Bead purified
60
42
30
22
HIS-tagged
GST-tagged

Fig. 5

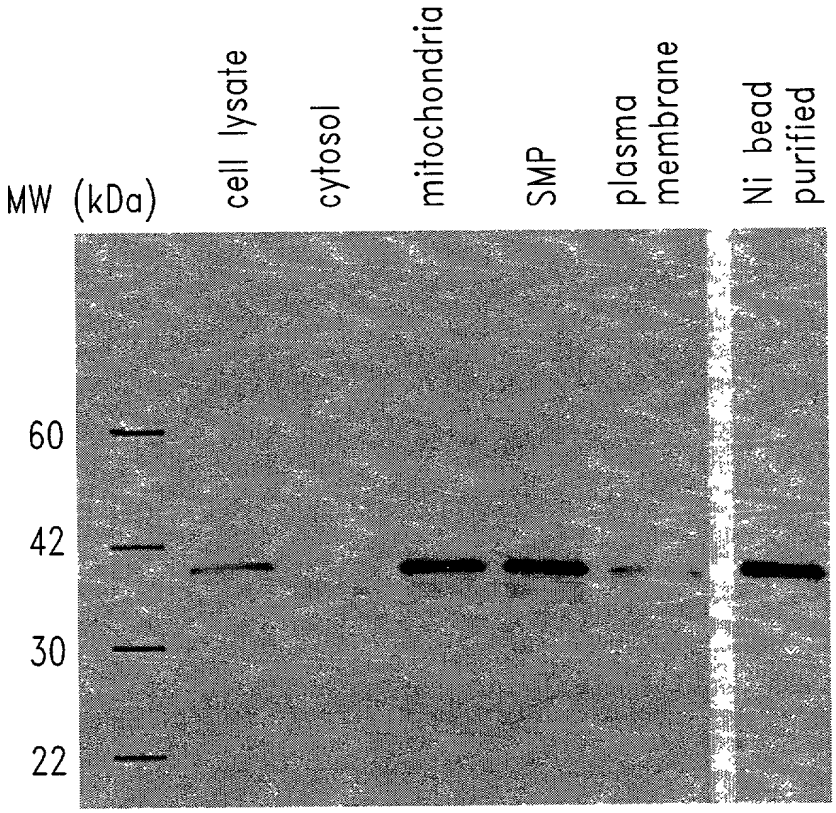
MW (kDa)
cell lysate
cytosol
mitochondria
SMP
plasma membrane
Ni bead purified
60
42
30
22

Fig. 6

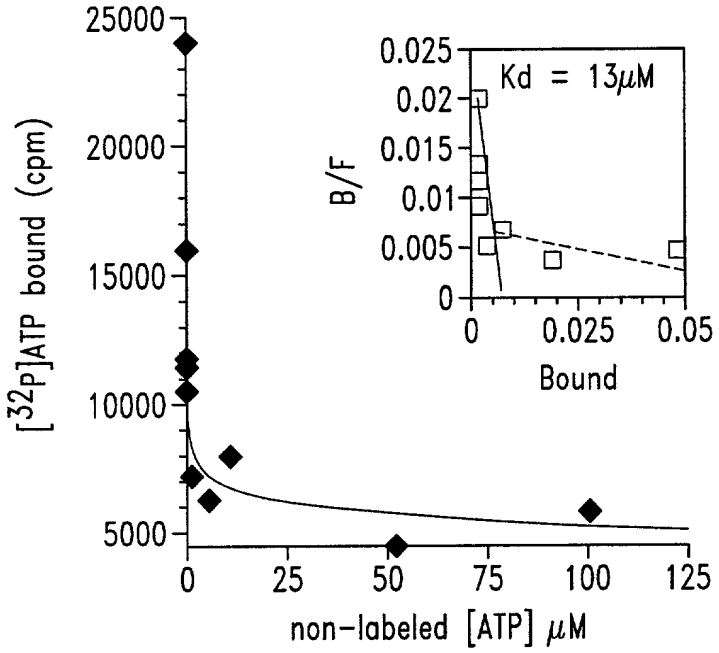
25000
20000
15000
10000
5000
[32P]ATP bound (cpm)
0
25
50
75
100
125
non-labeled [ATP] μM
0.025
0.02
0.015
0.01
0.005
0
B/F
Kd = 13μM
0
0.025
0.05
Bound

Fig. 7

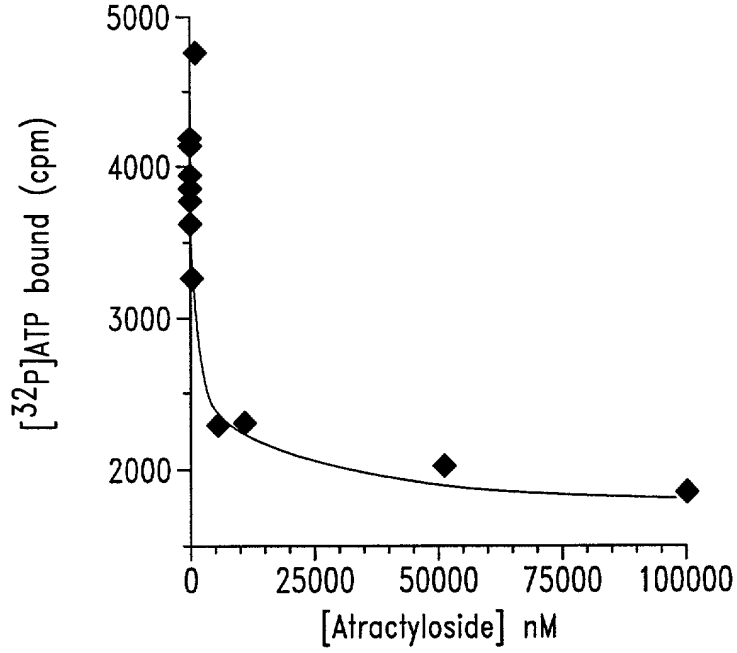
5000
4000
3000
2000
[32P]ATP bound (cpm)
0
25000
50000
75000
100000
[Atractyloside] nM

Fig. 8

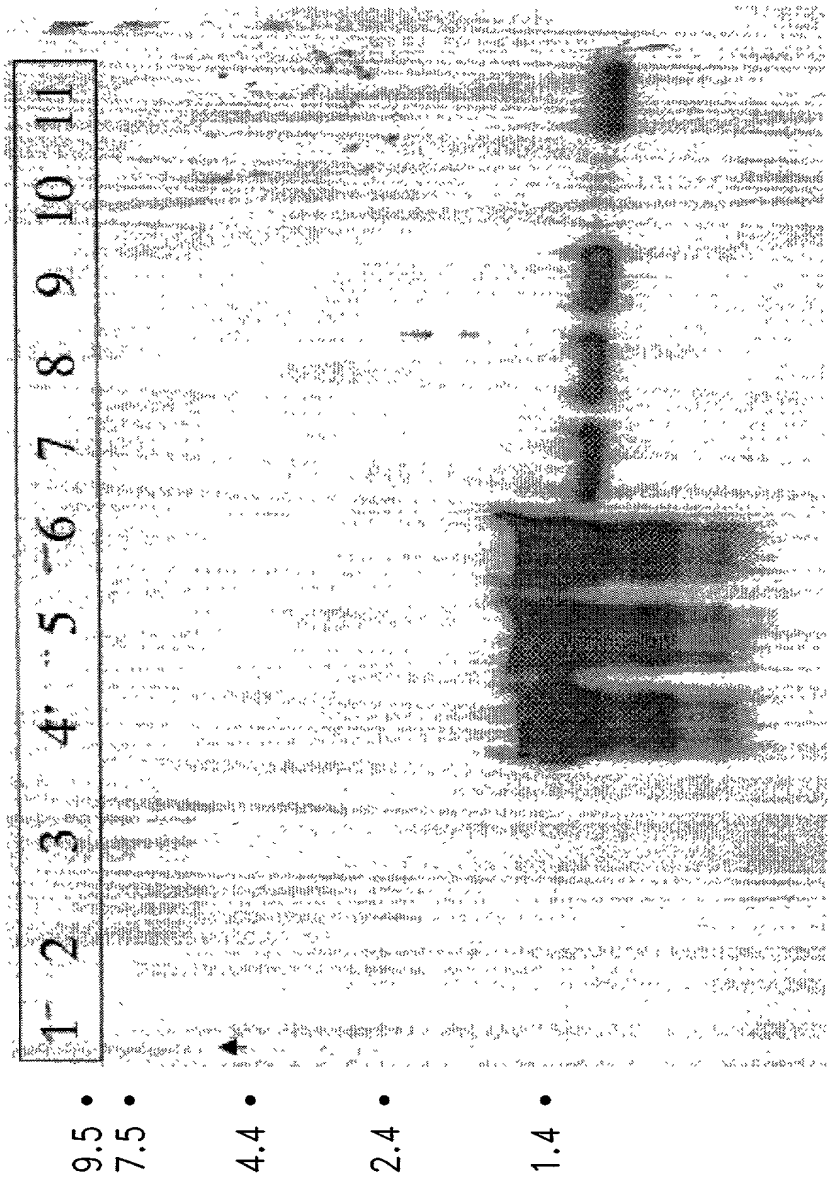

Fig. 10

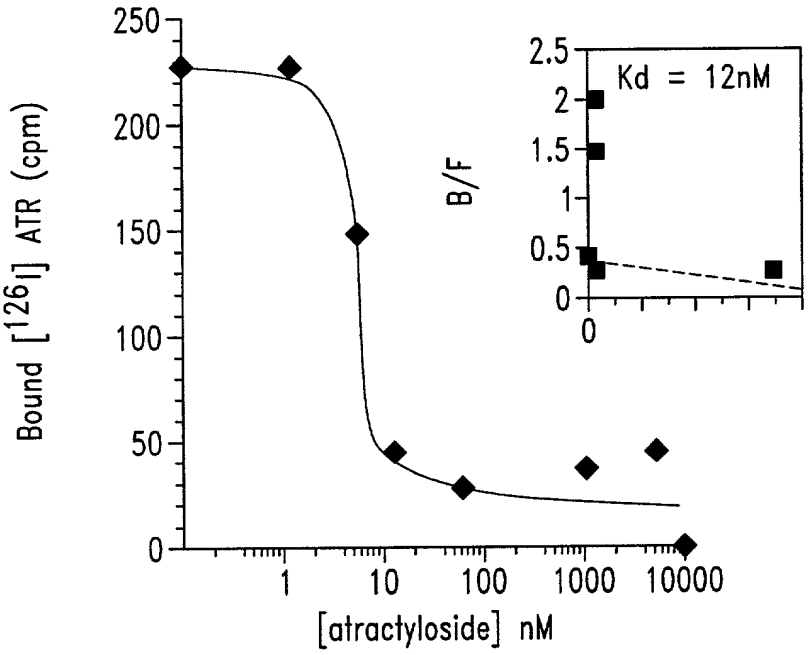
Bound [^{126}I] ATR (cpm)
250
200
150
100
50
0
1
10
100
1000
10000
[atractyloside] nM
B/F
2.5
2
1.5
1
0.5
0
0
Kd = 12nM

Fig. 9

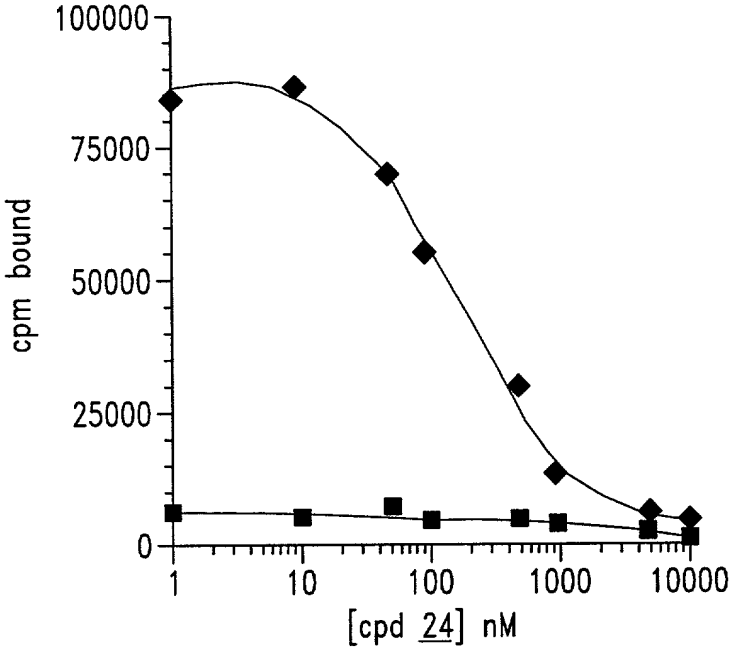
cpm bound
100000
75000
50000
25000
0
1
10
100
1000
10000
[cpd 24] nM

Fig. 11

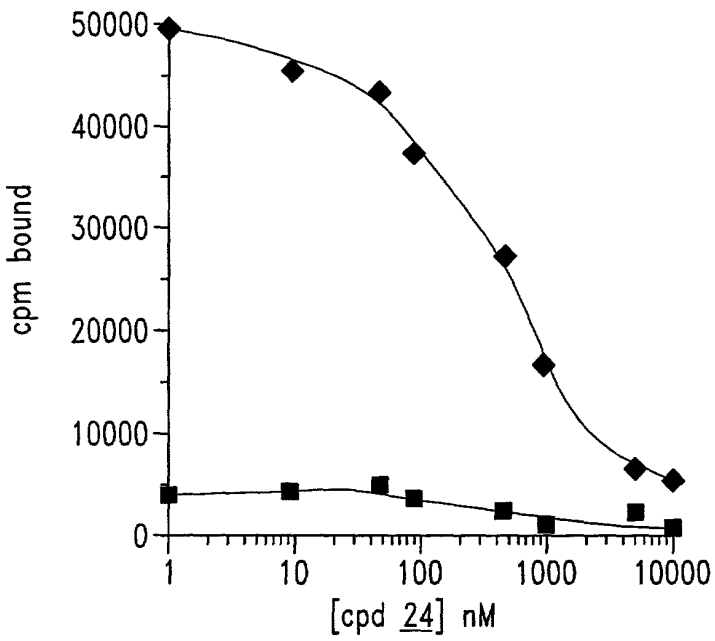
50000
40000
30000
20000
10000
0
cpm bound
1
10
100
1000
10000
[cpd 24] nM

Fig. 12

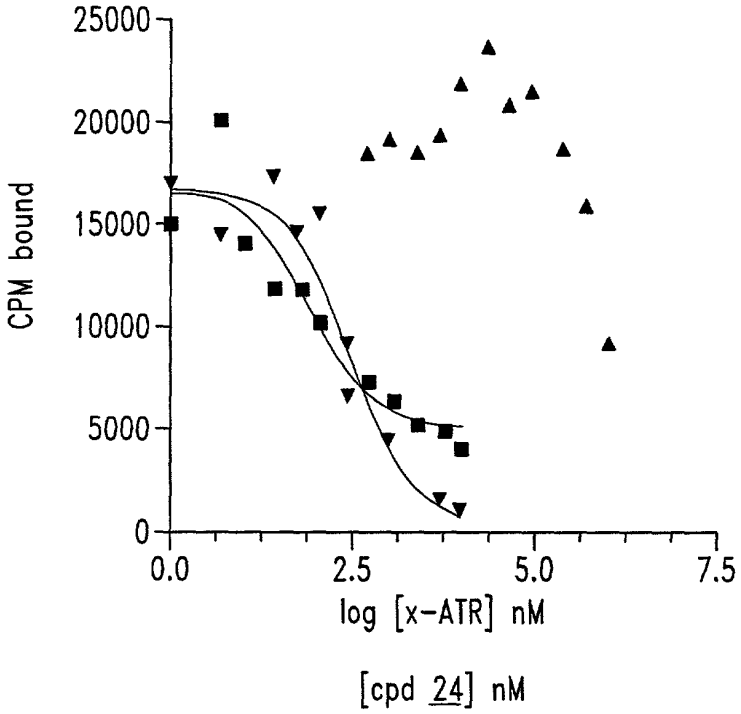
25000
20000
15000
10000
5000
0
CPM bound
0.0
2.5
5.0
7.5
log [x-ATR] nM
[cpd 24] nM

Fig. 13

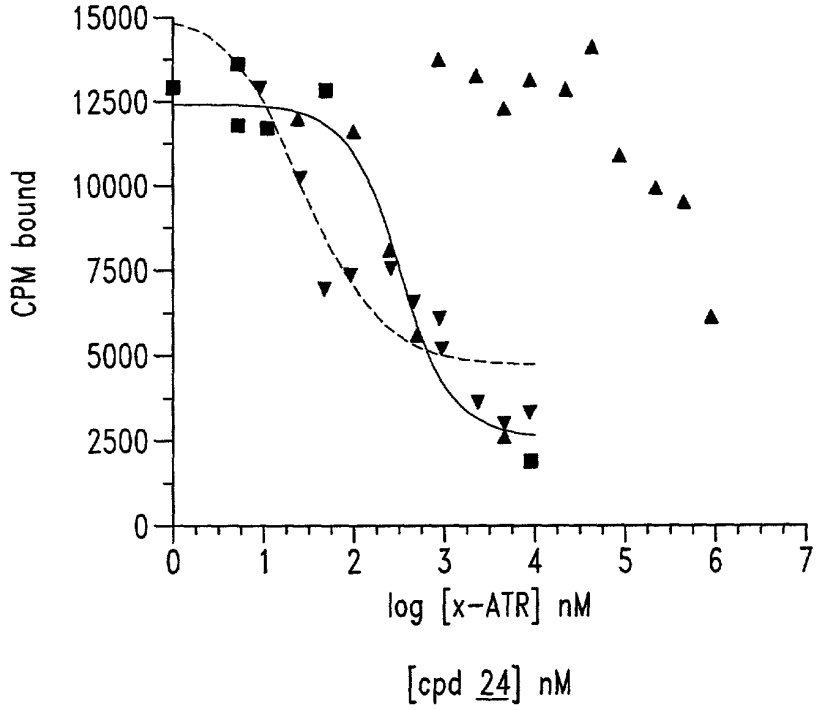
15000
12500
10000
7500
5000
2500
0
CPM bound
0
1
2
3
4
5
6
7
log [x-ATR] nM
[cpd 24] nM

Fig. 14

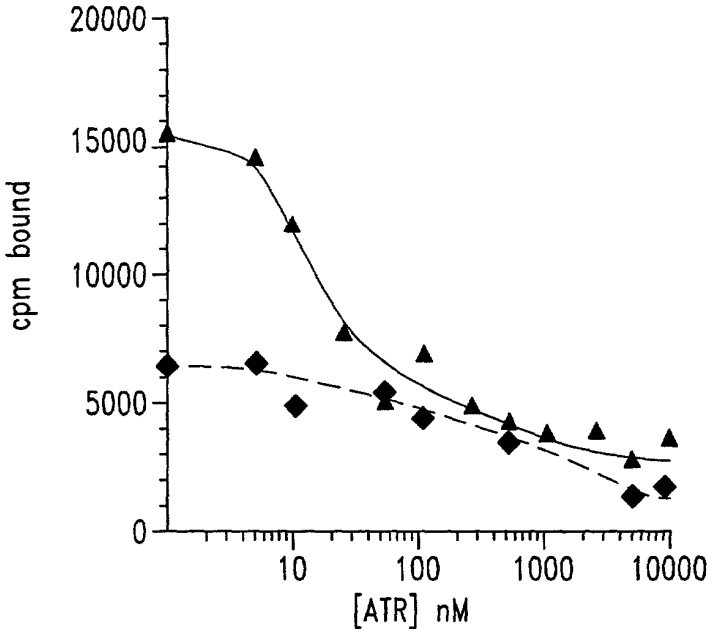
20000
15000
10000
5000
0
cpm bound
10
100
1000
10000
[ATR] nM

Fig. 15

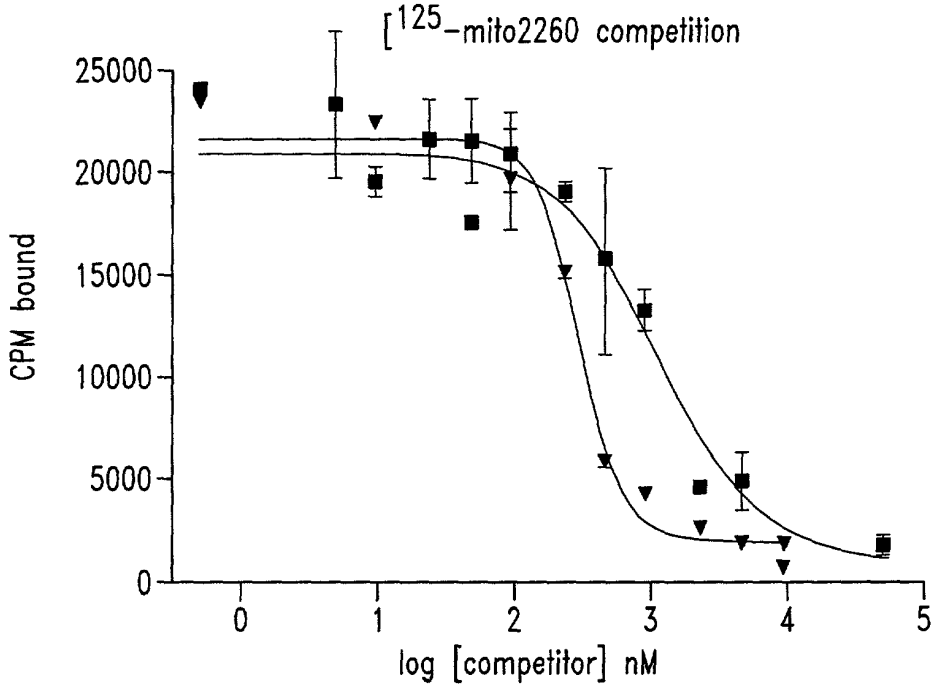
[125-mito2260 competition
CPM bound
25000
20000
15000
10000
5000
0
0
1
2
3
4
5
log [competitor] nM

Fig. 16

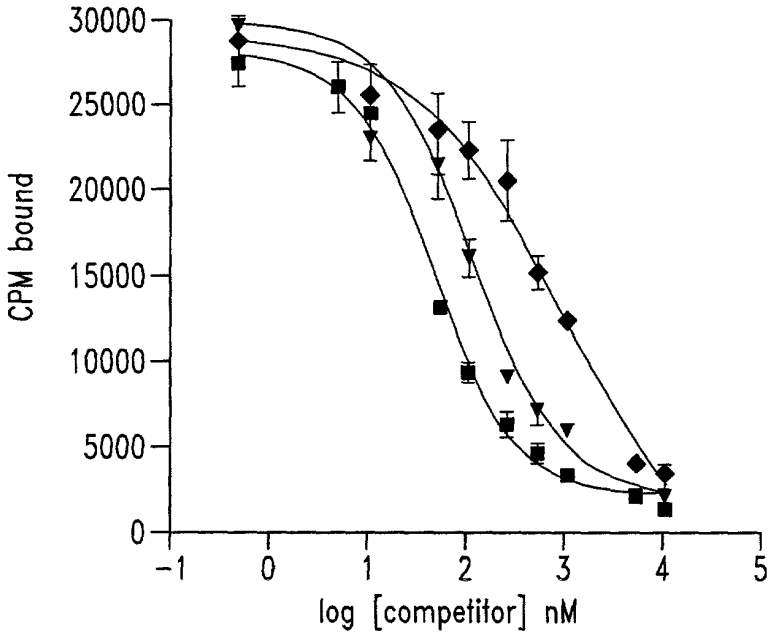
CPM bound
30000
25000
20000
15000
10000
5000
0
-1
0
1
2
3
4
5
log [competitor] nM

Fig. 17

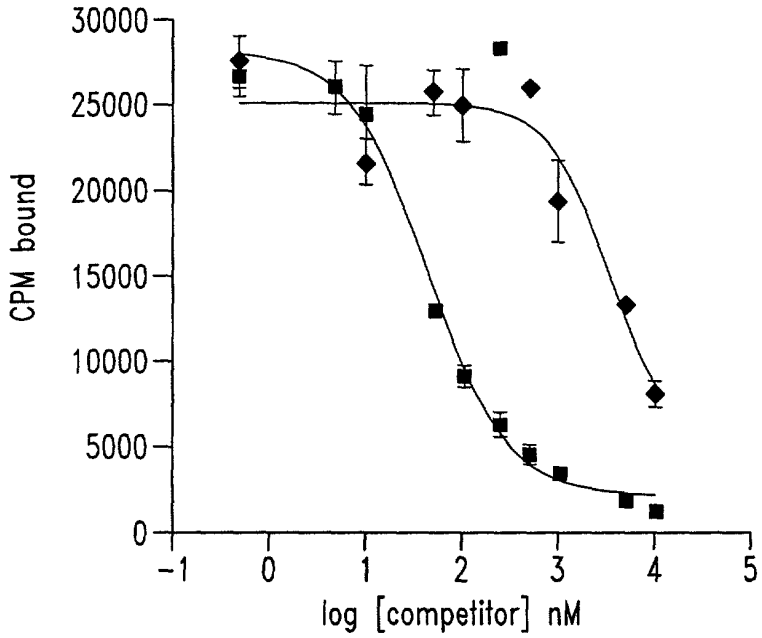
30000
25000
20000
15000
10000
5000
0
CPM bound
-1
0
1
2
3
4
5
log [competitor] nM

Fig. 18

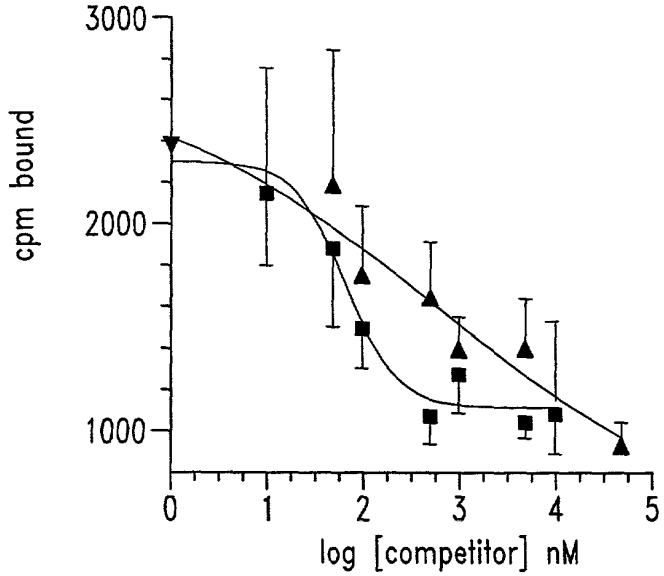
3000
2000
1000
cpm bound
0
1
2
3
4
5
log [competitor] nM

Fig. 19